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Pharmacokinetics of High-Dose Methotrexate in Egyptian Children with Acute Lymphoblastic Leukemia: Impact of Interpatient Variations

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Abstract :
Aim*:Since several factors have been shown to influence the clearance of methotrexate, the purpose of this study was to identify potential relationships between patient covariates and the methotrexate clearance estimates and deduce a pharmacokinetic model for the estimation of methotrexate clearance in Egyptian pediatric ALL patients that may help dosage adjustment and achieve target steady-state plasma concentrations in a similar sittings.*
Patients and methods: *A total of 94 pediatric patients with B-cell ALL, of whom 70 were the studied population and 24 were the test population, were treated with four courses of HDMTX doses 2.5 gm/m^2 (low-risk arm) or 5 gm/m^2 (standard-/high-risk arm) given every other week by intermittent intravenous infusions over 24 hours as a part of their treatment protocol. Patients were monitored for the 24 hour MTX concentration and the systemic methotrexate clearance was calculated for each methotrexate dose.*
Results:*The studied patients had average age of 7.1 ± 4.44 years; total body weight of 30.3 ± 21.04 kg, average height of 117.63 ± 28.09 cm, body surface area of 0.97 ± 0.45 m^2 and body mass index of 19.25 ± 4.49. The average creatinine clearance calculated using Schwartz formula was 167.83 ± 48.44 ml/min. The mean methotrexate clearance was 7.83 ± 4.52 L/hr. Estimated creatinine clearance and body mass index were identified as the most important factors influencing methotrexate clearance in the studied patients.*
Conclusion:*A Pharmacokinetic model was proposed to estimate the individual methotrexate clearance for Egyptian pediatric ALL patients. The derived final regression model reasonably predicted clearance values with best fitness and precessions.*

Keywords –*Pharmacokinetics, High-Dose Methotrexate, Childhood ALL, Steady state.*

I. INTRODUCTION

Oncology drugs have in general a narrower therapeutic index than drugs in common; this means that a minor change in dose may either result in severe toxicity, if increased, or poor anti neoplastic effects, if decreased. In both instances the consequences may be life threatening [1].

Childhood acute lymphoblastic leukemia (ALL) has a unique place in the history of oncology, as it was the first cancer to be cured by drugs. High-dose methotrexate (HDMTX) is an important element of chemotherapy in the treatment of childhood ALL and other malignancies [2]. Methotrexate doses higher than 1 gm/m^2 are beneficial for patients with B-lineage ALL [3]. However, high plasma methotrexate concentrations are also associated with increased toxicity which may delay subsequent courses of chemotherapy [2].

Pediatric ALL patients are subject to different pathological and physiological processes from those in the general population; from the presence of cancer-related antigens to anorexia and fluid challenges often given as part of chemotherapy schedules. Therefore administrations of doses to pediatric ALL patients that are based only on body surface area have frequently been associated with inappropriate concentrations [4].

Very little published information exists concerning methotrexate pharmacokinetics in Egyptian pediatric ALL patients and data concerning dosage adjustment depending on its pharmacokinetics principles are lacking. Accordingly, the investigation of the pharmacokinetic profile of methotrexate in Egyptian pediatric ALL patients and developing pharmacokinetic models based on patients' co-variables are considered important requirements to facilitate its dosage adjustment.

II. PATIENTS AND METHODS

2.1Study Design:

The present prospective cohort study was conducted at the Children's Cancer Hospital – Egypt (CCHE) 57357, Cairo, Egypt during the period between September 2013 and July 2014. The study was approved by the research and ethics committee of the faculty of pharmacy, Helwan University and the Institutional Review Board (IRB) of the local ethics committee of the CCHE 57357.

2.2Patient Selection:

Egyptian Pediatric children of either sex with ALL and under the age of 18 years old were the primary candidates for this study. A candidate was recruited when he or she had a provisional diagnosis of B-cell ALL confirmed by bone marrow aspirate and immunophenotyping (IPT) for leukemia. A written informed consent was obtained from the parents or from the children, when possible.

Patients were excluded from recruitment when they refused to sign the consent after explaining the nature and the steps of the study, their provisional diagnosis was not confirmed, or they had any contraindication to the study drug.

2.3 Test population:

Another group of patients, not belonging to the original patient population, who were admitted in the in-patient ward of CCHE 57357 with the same inclusion and exclusion criteria, were also recruited for validation of the results' equations.

2.4 Treatment protocol and drug administration:

The selected patients were treated with the Total XV chemotherapy protocol and the treatment was consisted of three main phases, Remission induction, Consolidation and Continuation.Remission-induction therapy included prednisone, vincristine, daunorubicin, L-asparaginase, cyclophosphamide, 6-mercaptopurine and cytarabine for 6 weeks. Risk classification was based on presenting characteristics and treatment response to remission-induction therapy, and patients were assigned to the low-, standard- or high-risk categories. The 6-week induction period was followed by consolidation phase, consisting of four courses of fixed HDMTX doses 2.5 gm/m^2 (low-risk arm) or 5 gm/m^2 (standard-/high-risk arm) given every other week together with triple intrathecal therapy with MTX, hydrocortisone and cytarabine on the day of MTX and daily oral 6-mercaptopurine at 50 mg/m^2/day at bedtime for the 8 weeks of consolidation.Continuation treatment begins 7 days after the fourth course of HDMTX of the consolidation treatment. Continuation treatment (120 days for girls and 146 days for boys) was consisted of dexamethasone, doxorubicin, vincristine, 6-mercaptopurine, L-asparaginase and methotrexate.

In the consolidation phase, Methotrexate (METHOTREXATE® 25mg/mL vial, Orion Corporation, Espoo, Finland) was given I.V. as a 24-hr infusion, where 10% of the dose was administered in 1 hr as a loading dose, and the remaining 90% in 23 hr(Target 24 hour MTX concentrations were 33 and 65 μmol/L for low-risk arm and standard-/high-risk arm, respectively). Infusion of MTX was preceded and accompanied by hyperhydration and alkalinization of urine by glucose 5% + NaCl 0.45% at a rate of 125 mL/m^2/hr with 25 mL 8.4% NaHCO3 and 5 mL KCl for each 500 mL. MTX infusion was started only when the urine pH was >6.5. Hyperhydration and alkalinization were continued until MTX plasma level reached an undetectable concentration (<0.3 μmol/L). Urine pH was monitored with each void and an IV sodium bicarbonate bolus was given if the urine pH was ≤ 6.Leucovorin rescue was started at 42 hr from the beginning of the MTX infusion. Those on the low-risk arm received 10 mg/m^2 of leucovorin I.V. every 6 hr for 5 doses and those on the standard-/high-risk arm received 15 mg/m^2 of leucovorin I.V. every 6 hr for 5 doses. Leucovorin doses were increased for patients with delayed excretion of methotrexate, defined as methotrexate concentrations >0.3 μmol/L at 42 hr. For those with delayed excretion, plasma methotrexate concentrations were monitored, and leucovorin was continued until MTX plasma level reached an undetectable concentration (<0.3 μmol/L).

2.5 Blood Sampling and Analysis:

Four blood samples for methotrexate serum determination, one sample for each methotrexate consolidation dose, were drawn from each patient at the end of the 24 hrinfusion.Methotrexate serum drug levels were measured by using enzyme multiple immunoassay technique (Emit® Methotrexate 2011), supplied by Siemens Healthcare Diagnostics Inc., SYVA®, USA. The sensitivity level of the Emit® Methotrexate Assay is 0.3 μmol /L methotrexate. This level represents the lowest concentration that can be distinguished from 0 μmol /L with a confidence level of 95%.

2.6Data Collection and Calculations:

Serum creatinine and blood urea nitrogen values were obtained prior to each methotrexate dose. Additional clinical factors such as estimated CrCl, body mass index (BMI) and body surface area (BSA) were calculated for each child according toSchwartz formula (1)[5],Eknoyan and Garabed formula (2)[6], and Mosteller formula (3)[7], respectively.Since the increase of plasma methotrexate concentration at 24 hours from the start of infusion is clearly dose related [8], the systemic methotrexate clearance (MTX Cl_T) will be calculated for each methotrexate consolidation dose from the infusion rate of methotrexate divided by the steady state concentration (Cpss) measured at the end of the 24-hr infusion (4)[9].

(1): CrCl (ml/min) = [K × Height (cm)] / Scr(mg/dl)

(2): BMI = Weight (kg) / Height (m) x Height (m)

(3): BSA (m^2) = ([Height (cm) x Weight (kg)]/ 3600)½

(4): $$\text{MTX Cl}_T\ (\text{L/hr}) = \frac{\text{MTX infusion rate } (\text{mg/m}^2\text{.hr}) \text{ x BSA } (\text{m}^2) \text{ x } 2.2}{\text{Cpss } (\mu\text{.mol/L})}$$

2.7 Statistical Analysis:

Analysis was conducted using SPSS version 22 and GraphPadInState software version 3.05. Results are presented as means ± standard deviations (SD) for quantitative measures.

Analysis of normality was performed using the Kolmogorov-Smirnov test. Friedman test was used for the comparison between the four methotrexate clearance values calculated after each methotrexate dose. The individual effect of gender was studied using Mann-Whitney U test.The individual effect of age, total body weight, height, body surface area, body mass index, creatinine clearance on methotrexate clearance was studied using simple linear regression analysis. Multiple regression analysis using GraphPadInStatesoftware was used to drive model describe the dependence of methotrexate clearance on the studied patients' covariates. Multicollinearity of variables were evaluated in the final model as each R squared quantifies how well each variable is predicted from the other variables (ignoring clearance) so that the variables of the final model should be independent of each other.To assess the validation of prediction of clearance values, Two-tailed student's t-test was used to examine the significance of any difference between the observed and predicted clearance values in the test population. P-values <0.05 were considered statistically significant.

III. RESULTS

3.1 Baseline Characteristics:

Seventy naïve Egyptian children with B-cell ALL were studied of whom 36 (51.43%) were males and 34 (48.57%) were females. The mean age for studied patients was 7.1 ± 4.44 years old. Serum creatinine (Scr), blood urea nitrogen (BUN) and estimated creatinine clearance (CrCl) using Schwartz formula were recorded before each HDMTX consolidation dose."Table 1" summarizes the baseline demographic and clinical data for the studied population.

3.2 Methotrexate Concentrations and Clearances:

A summary of methotrexate steady state concentrations (Cpss) and methotrexate clearances (MTX Cl_T),after each methotrexate consolidation dose, were presented in "Table 2". The mean methotrexate steady state levels were (59.7 ± 23.67), (51.67 ± 16.05), (52.79 ± 21.69) and (53.51 ± 21.68) µ.mol/L, and the mean methotrexate clearances were (6.88 ± 3.63), (7.84 ± 4.21), (8.35 ± 5.81) and (8.24 ± 5.78) L/hr in consolidation courses 1, 2, 3 and 4 respectively. There was a non-significant difference (p-value, 0.0701) between the calculated methotrexate clearances among the four methotrexate consolidation courses "Table 3 and Fig. 1"; so that an average methotrexate clearance for each patient was calculated to be used in the model building procedure. Also, an average creatinine clearance for each patient was calculated from the four estimated creatinine clearances to be used in the model building procedure.To simplify, the calculated average methotrexate clearance will be described as methotrexate clearance (MTX Cl_T) and the average estimated creatinine clearance will be described as creatinine clearance (CrCl) in the following study sections.

Table 1: Studied patients' baseline demographic and clinical features.

Characteristic	Studied patients(n = 70) mean ± S.D.	Range	CV%
Gender (Male/Female), %	36/34, 51.43% / 48.57%		
Risk Classification (Standard- and high-risk / low-risk), %	51/19, 72.86% / 27.14%		
Age (years)	7.1 ± 4.44	2 – 17	62.54%
TBW (kg)	30.3 ± 21.04	10.2 – 87	69.44%

Ht (cm)		117.63 ± 28.09	78 – 176	23.88%
BSA (m^2)		0.97 ± 0.45	0.48 – 2.05	46.39%
BMI		19.25 ± 4.49	12.39 – 30.48	23.32%
Scr (mg/dL)	Consolidation (1)	0.42 ± 0.06	0.3 – 0.6	14.29%
	Consolidation (2)	0.39 ± 0.05	0.2 – 0.6	12.82%
	Consolidation (3)	0.39 ± 0.03	0.3 – 0.5	7.69%
	Consolidation (4)	0.41 ± 0.06	0.3 – 0.7	14.63%
BUN (mg/dL)	Consolidation (1)	7.3 ± 5.9	3 – 16	80.82%
	Consolidation (2)	5.3 ± 2.21	3 – 9	41.7%
	Consolidation (3)	8.4 ± 3.21	4 – 13	38.21%
	Consolidation (4)	6.6 ± 4.1	3 – 15	62.12%
CrCl (mL/min)	Consolidation (1)	162.92 ± 51.89	100.1 – 302.75	31.85%
	Consolidation (2)	170.91 ± 53.61	100.1 – 308	31.37%
	Consolidation (3)	170.86 ± 51.64	107.25 – 308	30.22%
	Consolidation (4)	166.64 ± 47.6	107.25 – 308	28.56%

TBW: Total body weight;**Ht**: Height;**BSA**: Body surface area;**BMI:** body mass index;**Scr:** Serum creatinine;**BUN:** Blood urea nitrogen; **CrCl:** Estimated creatinine clearance using Schwartz formula; **S.D.:** Standard deviation, **CV%**: Coefficient of variation.

Table 2: Studied patients' methotrexate steady state levels and clearances.

Parameter	**Studied patients (n = 70) mean ± S.D.**	**Range**	**CV%**
Consolidation (1)			
Cpss (µ.mol/L)	59.7 ± 23.67	24 – 116	39.65%
MTX Cl_T (L/hr)	6.88 ± 3.63	2.49 – 16.66	52.76%
Consolidation (2)			
Cpss (µ.mol/L)	51.67 ± 16.05	11 – 89	31.1%
MTX Cl_T (L/hr)	7.84 ± 4.21	2.71 – 20.15	53.7%
Consolidation (3)			
Cpss (µ.mol/L)	52.79 ± 21.69	18 – 148	41.1%
MTX Cl_T (L/hr)	8.35 ± 5.81	2.34 – 26.85	69.6%
Consolidation (4)			
Cpss (µ.mol/L)	53.51 ± 21.68	19 – 124	40.5%
MTX Cl_T (L/hr)	8.24 ± 5.78	2.08 – 27.64	70.1%

Cpss: Methotrexate steady state concentration;**MTX Cl_T:** Methotrexate clearance;**S.D.:** standard deviation;**CV %:** percent coefficient of variation.

Table 3: The calculated methotrexate clearances among the four consolidation courses

Parameter	**Consolidation course (n = 70)**				***P-value*[§]**	**Sig.**
	(1)	**(2)**	**(3)**	**(4)**		
MTX Cl_T (L/hr) Mean ± S.D. Range	6.88 ± 3.63 2.49 – 16.66	7.84 ± 4.21 2.71 – 20.15	8.35 ± 5.81 2.34 – 26.85	8.24 ± 5.78 2.08 – 27.64	0.0701	>0.05 ns

[§] **(Friedman test)** (**MTX Cl_T:** Methotrexate clearance; **S.D.:** Standard deviation; **ns:** non- significant).

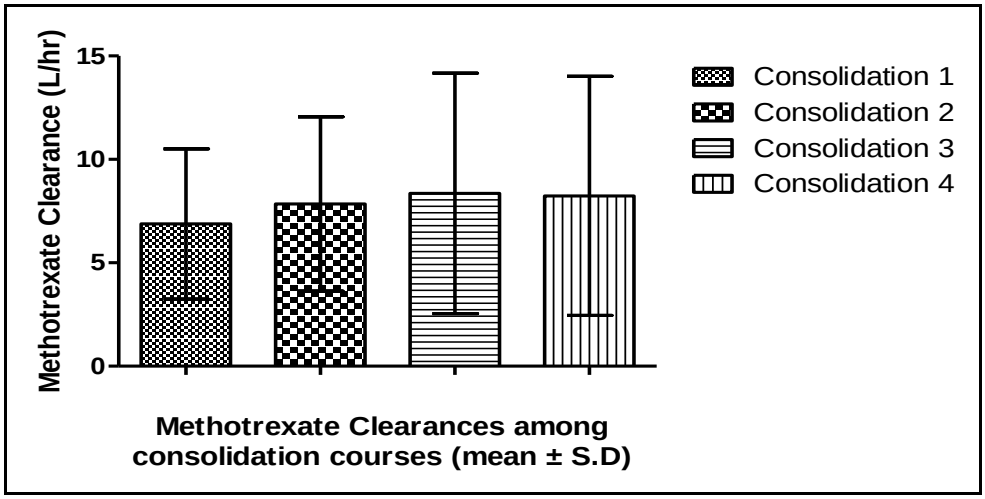

Figure 1: Methotrexate clearances among the four consolidation courses

3.3 Effect of the Studied Patients Co-variables on Methotrexate Clearance (MTX Cl_T):

The covariates evaluated were gender, age, weight, height, body surface area (BSA), body mass index (BMI) and creatinine clearance (CrCl).

Gender in the studied patients showed a non-significant effect (p-value, 0.6510) on methotrexate clearance comparing males with females. The mean clearance was 8.17 ± 5.07 L/hr in male group and 7.46 ± 3.9 L/hr in female group. The linear regression plots of methotrexate clearance versus other covariates indicated that there was a significant increase (p-value < 0.0001) in methotrexate clearance with increasing age, weight, height, body surface area, body mass index and creatinine clearance "Table 4 and Fig. 2".

3.4 Building of the Methotrexate Clearance Model:

The basic model was created by incorporating all covariates that have significant effect on methotrexate clearance. The influence of age, body weight, height, body surface area, body mass index and creatinine clearance were tested and the strength of these relationships was shown by the basic model of clearance (5) (R^2 value 95.38%; p-value <0.0001). Evaluation of the effect of removal of each covariate from the basic model of methotrexate clearance revealed that age, body mass index and creatinine clearance were the covariates of the significant contribution "Table 5".Thereby a reduced form of the basic model was deduced (6) (R^2 value 93.09%; p-value <0.0001). Upon evaluation of the significance of the covariates, body mass index and creatinine clearance were the covariates of the significant contribution in the reduced model "Table 5".Age showed a non-significant relationship with methotrexate clearance in the reduced model so that it was not combined into the final model (7) (R^2 value 92.71%; p-value <0.0001). Both body mass index and creatinine clearance were significant contributors in the final model "Table5".According to "Table 6", all R squared values were low (<0.75), so the variables of the final model were independent of each other andmulticollinearity was not a problem.

(5): MTX Cl_T (L/hr)= −5.017—0 .1339*[Age (years)] + 0.1932*[Weight (kg)] + 0.04850*[Height (cm)] — 5.064*[BSA (m^2)] + 0.04961*[BMI] + 0.03699*[CrCl (mL/min)]

(6): MTX Cl_T (L/hr)= − 8.336 + 0.1449*[Age (years)] + 0.2502*[BMI] + 0.06148*[CrCl (mL/min)]

(7): MTX Cl_T (L/hr)= −9.597 + 0.2830*[BMI] + 0.07136*[CrCl(mL/min)]

Table 4: Summary of the effect of studied patient's co-variables on methotrexate clearance

Parameter	**Mean ± SD Range**		***r****	**r squared**	***P* value**	***Sig.***
Gender	Male 8.17 ± 5.07 2.89 – 22.11	Female 7.46 ± 3.9 2.88 – 17.19			0.6510	>0.05 ns
Age (years)	7.1 ± 4.44 2 – 17		0.8952	0.8014	<0.0001	<0.05 significant

Weight(kg)	30.3 ± 21.04 10.2 – 87	0.9606	0.9227	<0.0001	<0.05 significant
Height (cm)	117.63 ± 28.09 78 – 176	0.9331	0.8707	<0.0001	<0.05 significant
BSA (m^2)	0.97 ± 0.45 0.48 – 2.05	0.9645	0.9304	<0.0001	<0.05 significant
BMI	19.25 ± 4.49 12.39 – 30.48	0.7509	0.5639	<0.0001	<0.05 significant
CrCl (mL/min)	167.83 ± 48.44 107.25 – 292.6	0.9370	0.8780	<0.0001	<0.05 significant

***r*:** correlation coefficient;**BSA:** body surface area;**BMI:** body mass index;**CrCl:** estimated creatinine clearance

Table 5: Contribution of different patients' co-variables in the basic, reduced and finalMTX Cl_Tmodels

MTX Cl_T Model		**Studied patients' co-variables (n = 70)**						
		Constant	**Age**	**Weight**	**Height**	**BSA**	**BMI**	**CrCl**
Basic Model	p-value	0.0210	0.0161	0.1281	0.4978	0.6401	0.0497	<0.0001
	Significant?	Yes	Yes	No	No	No	Yes	Yes
Reduced Model	p-value	<0.0001	0.0631	----------	----------	----------	<0.0001	<0.0001
	Significant?	Yes	No	----------	----------	----------	Yes	Yes
Final Model	p-value	<0.0001	----------	----------	----------	----------	<0.0001	<0.0001
	Significant?	Yes	----------	----------	----------	----------	Yes	Yes

MTX Cl_T: methotrexate clearance;**BSA:** body surface area;**BMI:** body mass index; **CrCl:** estimated creatinine clearance

Table 6: Multicolinearity data of the finalMTX Cl_Tmodel

Variable	**R^2 with other X**
BMI	0.3780
CrCl	0.3780

BMI: body mass index; **CrCl:** estimated creatinine clearance

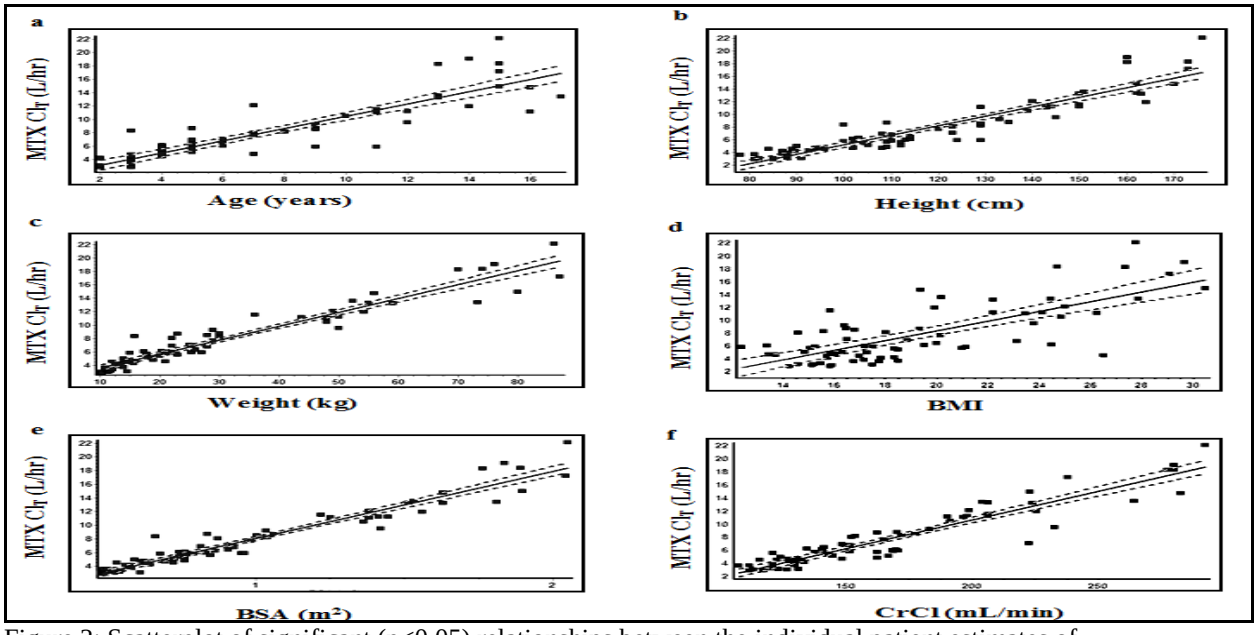

Figure 2: Scatterplot of significant (p<0.05) relationships between the individual patient estimates of methotrexate clearance (MTX Cl_T) versus covariates

3.5 Validation of the Final MTX Cl_T Model:

Validation of the final methotrexate clearance model was performed on the test population.These additional patients were 24 of whom 13 (54.2%) were males and 11 (45.8%) were females.The validation procedure was done for each methotrexate consolidation dose using patients' BMI and CrCl to assess the use of the final model in prediction of methotrexate clearance in all consolidation courses. The paired-comparison t-test between the observed and predicted methotrexate clearance values of the final model among the four methotrexate consolidation courses were considered not significant (p-values were 0.6667, 0.1861, 0.0892 and 0.4860 in consolidation courses 1, 2, 3 and 4; respectively) "Table 7".

Table 7: Paired-comparison t-test between observed and predicted methotrexate clearance among the four consolidation courses

Consolidation	**Test population (n = 24)**		***Two tailed P-value*[§]**	***Sig.***
	Observed MTX Cl_T (L/hr) Mean ± S.D	**Predicted MTX Cl_T (L/hr) Mean ± S.D**		
(1)	8.34 ± 4.27	8.54 ± 3.9	0.6667	>0.05 ns
(2)	9.22 ± 4.63	9.73 ± 5.23	0.1861	>0.05 ns
(3)	9.53 ± 5.23	9.15 ± 5.37	0.0892	>0.05 ns
(4)	10.01 ± 7.62	9.57 ± 5.21	0.4860	>0.05 ns

[§] (Paired t test), (**MTX Cl_T:** Methotrexate clearance;**S.D.:** Standard deviation;**ns:** non- significant).

IV. DISCUSSION

HDMTX is an important chemotherapeutic agent that contributes to the high cure rate of pediatric ALL [10, 11]. Higher plasma methotrexate concentrations following HDMTX have been associated with a lower risk of relapse [12]. A standard fixed MTX dose can produce up to a 7-fold spread in the range of drug concentration in different patients [13]. Consequently, MTX is the cytotoxic drug subjected to the most extensive plasma concentration monitoring in clinical practice. In fact, the benefit of applying therapeutic drug monitoring (TDM) in the individualization of high-dose MTX therapy in all children has been demonstrated [14, 15].

Traditionally the dosing of most anti-cancer drugs, including methotrexate, is individualized according to body surface area (BSA) [16]. Modification according to body size is especially indicated when it varies greatly, as during growth, but the quest for other ways to tailor doses is also desired since much of the variability is still unexplained [17]. Although BSA dose modifications were useful to predict a safe starting dose in the first human studies with a new chemical entity, it is not clear, for many clinical practice, why to keep continue the same approach in spite of very few physiological factors relevant to pharmacokinetics are related to BSA [4].

It has been shown that adjusting the dose of MTX, to account for inter-individual differences in drug clearance and to achieve a target Cpss, improved the outcome in children with B-cell ALL [15, 18, 19] and using prior information about a patient's MTX clearance to individualize patient's dosage of HDMTX is an added way to potentially reduce adverse effects [2].

Our study has examined the drug levels and clearance of methotrexate in pediatric ALL Egyptian patients using blood samples collected as a part of the routine monitoring. Although methotrexate disposition can be described and its serum concentrations fitted using a two- or three-compartment pharmacokinetic model, this is not typically done in the clinical setting since more intensive sampling is required [20]. We collected samples suitable for analysis using a one-compartment model so that our results would be of direct utility to clinical practitioners who use this method to estimate methotrexate clearance. The relevant parameters for dosing can be accurately characterized using a one-compartment model if the plasma concentration is drawn after the initial distribution phase is completed [8, 9].

In the current study, a methotrexate clearance model was built from 280 HDMTX courses in 70 children diagnosed with B-cell ALL that were recruited from the in-patient ward of the Children's Cancer Hospital – Egypt (CCHE) 57357, Cairo, Egypt. They were all of an Egyptian origin in order to avoid any variability in methotrexate drug levels and clearance due to ethnic disparities. MTX doses were given as a continuous I.V infusion over 24

hours as a part of the Total XV protocol and the systemic methotrexate clearance (MTX Cl_T) was calculated for each methotrexate dose from the infusion rate of methotrexate divided by the steady state concentration (Cpss) measured at the end of the 24-hr infusion [9].Since, the pharmacokinetic parameters of methotrexate, reported in several previous studies for children with ALL receiving high-dose methotrexate, have been calculated using classical approaches [14, 15, 20-25] comparison of these parameters with those employed in this study may be appropriate.

In our study the mean MTX Cl_T obtained among the four HDMTX doses correspond well to those of other investigators. Thus, the mean MTX Cl_T (7.83 L/hr) lies within the previously reported range (3.5 – 8.8) when clearance is not normalized by TBW or BSA [25-29]. Some studies have reported a dose dependency of methotrexate pharmacokinetics, arising from the saturation of its metabolism and responsible for a decrease in clearance with increasing doses [21]. Thus, the lowest MTX Cl_T found by Odoul et al (3.5 L/hr) could be attributable to the higher dosage (8gm/m^2). It should be noted that the study of Odoul et al [27] was conducted on 23 ALL pediatric patients, age range from 9 months to 15 years, and the doses of HDMTX in the consolidation phase were given every 2 weeks as a loading dose of 1.6gm/m^2 over 0.5 hour followed by the rest of the dose 6.4gm/m^2 over 23.5 hours.

In general, most studies show that body size, measured as TBW or BSA, influences the clearance of methotrexate, which are often normalized by kg or m^2[21-25]. In children, the close relationship among covariates representative of body size prevents the possibility of including all of them in the pharmacostatistical model, despite their individual correlation with the pharmacokinetic parameters. Instead, only one of the body size indices should be retained in the final model. Although the use of BSA in methotrexate dosage is standard practice, our results indicate that BMI and estimated CrCl are the best predictors of MTX Cl_T.

MTX Cl_T in this study was found to have a significant correlation with patients' age (p-value < 0.0001); this was in agreement with other investigators who have also found significant differences in methotrexate pharmacokinetic behavior with age [15, 21, 27]. Since age in children is closely related to BMI, the influence of both covariates is redundant; therefore according to our results age was not found in the final clearance model as a continuous covariate. These results agree with those reported in Aumente et al [20], whose study was performed on 49 ALL pediatric patients, age ranged from 6 months to 17 years, and the MTX doses were 3gm/m^2 infused over 24 hours with 10% of the dose infused over 0.5 hour and the rest infused over 23.5 hours.

The association between low urine pH and high risk methotrexate concentrations has been well documented and measurement of low urine pH values during methotrexate infusion has been found to be significantly associated with delayed excretion [30]. In the current study, urine pH is strictly controlled during therapy which made it difficult to include in the basic clearance model since the time it remains low values is unknown.

The sex in this study was not included in the basic clearance model because it was not found to have a significant effect (p-value 0.6510) on MTX Cl_T. Wall et al. found a lower methotrexate clearance in females than in males (p-value < 0.03) [15], although other previous studies [27, 31] have not reported sex differences in HDMTX clearance.

It has been also reported that renal function plays a key role in methotrexate pharmacokinetics and a delayed elimination of methotrexate appears to be related to elevated serum creatinine and decreased creatinine clearance [32, 33]. Methotrexate itself can cause acute nephrotoxicity and close monitoring of patients receiving methotrexate is imperative. At CCHE 57357, close monitoring of fluid status, urine output, urine pH, laboratory values, methotrexate concentrations [2, 31] and drug interactions, in order to prevent delayed excretion and toxicity, are performed with each course of HDMTX. Close monitoring allows early intervention (e.g., increasing fluid hydration, discontinuing interacting drugs) and potentially reduces adverse effects.In the present study, serum creatinine and estimated CrCl using Schwartz formula were selected as indicators of renal function in the studied subjects. However, the initial indices of renal function in the patients included in the present analysis were close to normal values and no important changes were observed throughout the therapy, it should be noted that methotrexate clearance was significantly increased with increase in estimated CrCl (p-value < 0.0001). This was in agreement with the study of Skarby et al. [33]. As a result, estimated CrCl was incorporated in the basic model and continued to the final clearance model.

In the present study, the predictive performance of the final proposed methotrexate clearance model was evaluated in the test population using paired comparison t-test to examine the significance of difference between the observed and predicted methotrexate clearance values among the four methotrexate consolidation courses. This validation methodology was previously used by other investigators [34, 35].Current study results of paired comparisons between the observed and predicted methotrexate clearance values of the final model among the four methotrexate consolidation courses were considered not significant. These results suggest that the derived final regression model of methotrexate clearance, **[MTX ClT (L/hr)]= − 9.597 + 0.2830*[BMI] + 0.07136*[CrCl (mL/min)]** (R^2 value 92.71%; p-value <0.0001), reasonably predicted clearance values and could be used in methotrexate dosage adjustment to achieve target steady state plasma concentrations.

V. CONCLUSION

There was a significant individual variability in methotrexate serum concentrations and clearance in the studied Egyptian pediatric ALL patients. Estimated creatinine clearance and body mass index were identified as the most important factors influencing methotrexate clearance in the studied patients.A Pharmacokinetic model was proposed to estimate the individual methotrexate clearance for Egyptian pediatric ALL patients. The derived final regression model reasonably predicted clearance values with best fitness and precessions. Methotrexate dosage adjustment to achieve target steady state plasma concentration can be done using this validated equation between methotrexate clearance and patient specific data.

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